Bioavailability of everolimus administered as a single 5 mg tablet versus five 1 mg tablets: a randomized, open-label, two-way crossover study of healthy volunteers

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Background: The mammalian target of rapamycin (mTOR) inhibitor everolimus has a well-established pharmacokinetics profile. We conducted a randomized, single-center, open-label, two-sequence, two-period crossover study of healthy volunteers to assess the relative bioavailability of everolimus administered as one 5 mg tablet or five 1 mg tablets.

Methods: Subjects were randomized 1:1 to receive everolimus dosed as one 5 mg tablet or as five 1 mg tablets on day 1, followed by a washout period on days 8–14 and then the opposite formulation on day 15. Blood sampling for pharmacokinetic evaluation was performed at prespecified time points, with 17 samples taken for each treatment period. Primary variables for evaluation of relative bioavailability were area under the concentration–time curve from time zero to infinity (AUCinf) and maximum blood concentration (Cmax). Safety was assessed by reporting the incidence of adverse events (AEs).

Results: Twenty-two participants received everolimus as one 5 mg tablet followed by five 1 mg tablets (n=11) or the opposite sequence (n=11). The Cmax of five 1 mg tablets was 48% higher than that of one 5 mg tablet (geometric mean ratio, 1.48; 90% confidence interval [CI], 1.35–1.62). AUCinf was similar (geometric mean ratio, 1.08; 90% CI, 1.02–1.16), as were the extent of absorption and the distribution and elimination kinetics. AEs, all grade 1 or 2, were observed in 54.5% of subjects.

Conclusion: Although the extent of absorption was similar, the Cmax of five 1 mg tablets was higher than that of one 5 mg tablet, suggesting these formulations lead to different peak blood concentrations and are not interchangeable at the dose tested.

Keywords: absorption kinetics, healthy volunteers

Introduction

The mammalian target of rapamycin (mTOR) signaling pathway is important in the regulation of cell growth, proliferation, metabolism, survival, and angiogenesis.¹² Because activation of mTOR is commonly associated with the pathogenesis of multiple tumor types, inhibition of the pathway provided the rationale for the development of anticancer therapies targeting mTOR.³ Everolimus, an oral mTOR inhibitor, has been studied extensively in patients with multiple tumor types and tuberous sclerosis complex (TSC).³ In addition to its clinical profile, the pharmacokinetics (PK) and pharmacodynamics profiles of everolimus have been well characterized in patients with advanced solid tumors and TSC.⁴⁵ Results of a Phase I study conducted in patients with advanced solid tumors demonstrated sustained activity over 7 days at oral everolimus
doses ≥20 mg once weekly.3 Area under the concentration–
time curve (AUC) was dose-proportional, but maximum 
blood concentration (Cmax) increased less than proportion-
ally at doses ≥20 mg once weekly. Everolimus ≥20 mg 
once weekly or ≥5 mg once daily was recommended as the 
optimal effective dose. Results of another Phase I study of 
oral everolimus demonstrated more profound and sustained 
mTOR pathway-inhibition with everolimus 10 mg once daily 
than with 50 mg once weekly.4

Everolimus (Afinitor®; Novartis Pharmaceuticals Corp, 
East Hanover, NJ, USA) is approved in various countries for the 
treatment of patients with advanced hormone receptor-positive, 
HER2-negative breast cancer, in combination with exemestane 
after failure of letrozole or anastrozole; progressive, advanced 
pancreatic neuroendocrine tumors; renal cell carcinoma after 
failure of prior sunitinib or sorafenib; renal angiomylipoma 
associated with TSC not requiring immediate surgery; and 
subependymal giant cell astrocytoma associated with TSC 
not amenable to curative resection.8 As Afinitor, everolimus 
is available as 2.5 mg, 5 mg, 7.5 mg, and 10 mg tablets, and 
as 2 mg, 3 mg, and 5 mg dispersible tablets for oral suspen-
sion (Afinitor Disperz; Novartis Pharmaceuticals Corp).8 
Under the trade names Certican® (Novartis Pharma AG, 
Basel, Switzerland) and Zortress® (Novartis Pharmaceuticals 
Corp), everolimus has been approved for the prophylaxis 
of organ rejection in adult patients who received a renal, 
hepatic, or cardiac transplant and is available in formulations 
of 0.25 mg, 0.5 mg, 0.75 mg, and 1 mg tablets, and 0.1 mg 
and 0.25 mg dispersible tablets.9 Everolimus 1 mg tablets have 
not been studied in the oncology setting. Although 1 mg and 
5 mg everolimus tablets have the same active ingredient and 
excipients, the proportional composition of the components 
differs between formulations. It is also not known whether 
some 1 mg tablets deliver the same peak concentration and 
bioavailability as a single higher dose tablet.

The purpose of this study was to determine the relative 
bioavailability of a single 5 mg oral dose of everolimus 
administered as one 5 mg tablet (reference) or as five 1 mg 
tables (test). The secondary objective was to evaluate the 
safety and tolerability of both formulations.

Subjects and methods
Subjects
Male or female volunteers between 18 and 65 years of age 
who were in good health, as determined by medical his-
tory, current medical condition, physical examination, vital 
signs, electrocardiogram (ECG), and laboratory tests, were 
eligible for inclusion in the study. Pregnant or lactating 
mothers and women of childbearing age and sexually 
active men (unless they were using contraception during 
and 8 weeks after dosing) were ineligible. Other exclusion 
criteria included tobacco or nicotine use within 3 months 
before screening; use of any prescription drugs within 
30 days before dosing or over-the-counter medication or 
herbal supplement within 14 days of baseline assessments; 
participation in any clinical investigation within 4 weeks of 
dosing; known hypersensitivity to rapamycin and its deriva-
tives; and a positive test for HIV antibody, hepatitis B surface 
antigen, or hepatitis C antibody. Use of CYP3A4 and/or 
P-glycoprotein inhibitors, inducers, or substrates was pro-
hibited in the 30 days before the first everolimus dose until 
the end of the study. Consumption of grapefruit (and juice), 
star fruit (and juice), and cruciferous vegetables within 7 days 
of baseline assessments and of caffeine within 12 hours of 
baseline assessments was also prohibited.

The study protocol was approved by Ethics Committee 
of the Land Berlin, State Office of Health and Social Affairs 
Berlin, Berlin, Germany. The study was conducted in accor-
dance with the ICH Harmonized Tripartite Guidelines for 
Good Clinical Practice with applicable local regulations, and 
with the ethical principles laid down in the Declaration of 
Helsinki. All participants provided written informed consent 
before screening.

Study design
This Phase I randomized, single-center, open-label, two-
sequence, two-period crossover study was conducted in 
healthy volunteers (Figure 1). After a 14-day screening period, 
participants were randomly assigned 1:1 to receive a single 5 
mg everolimus tablet on day 1, followed by a washout period 
on days 8–14 and then five 1 mg everolimus tablets on day 15, 
or the opposite sequence. All everolimus doses were admin-
istered with 240 mL of water under the supervision of study 
personnel, under fasting conditions. Participants received a 
standardized, light, low-fat dinner at least 10 hours before 
study drug administration; after this, no food was allowed until 
at least 4 hours postdose, when a standard meal was served. 
Meals were controlled for up to 72 hours postdose. Aside 
from water for dosing, one fluid was permitted within 1 hour 
before and after dosing. Water was allowed ad libitum at all 
other times. End-of-study evaluation was conducted 14 (±2) 
days after everolimus administration in the second treatment 
period. Subjects were discontinued from the study in the event 
of grade 3 or 4 adverse events (AEs), abnormal laboratory 
values or test procedures, or protocol deviation. Subjects could 
voluntarily withdraw from the study at any time.
Pharmacokinetic assessments
Blood sampling for PK evaluations was performed at pre-specified time points, with 17 samples collected during each treatment period: one predose sample collected up to 24 hours before everolimus administration and 16 postdose samples collected 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 24, 48, 72, 96, 120, and 144 hours after everolimus administration. At each time point, 2 mL venous blood was collected into a tube containing dipotassium ethylenediaminetetraacetic acid (K₂EDTA) anticoagulant and stored at −20°C or below within 60 minutes of the draw time. Everolimus concentration in whole blood was determined at a central laboratory (WuXi AppTec, Shanghai, China) by a validated method of liquid chromatography mass spectrometry (LC-MS/MS) with solid-phase extraction (lower limit of quantification [LLOQ], 0.300 ng/mL).

Primary variables for evaluation of relative bioavailability were AUC from time zero to infinity (AUCinf) and Cmax. Secondary variables were AUC from time zero to the last observation time point, regardless of whether that concentration is quantifiable (AUCall), AUC from time zero to the 144-hour concentration sampling time (AUC0–144h), time to reach Cmax (Tmax), terminal slope of elimination phase, apparent volume of distribution (Vd/F), total body apparent clearance of drug from the blood (CL/F), elimination half-life (T1/2), and mean resident time (MRT). All PK parameters were analyzed using Phoenix® WinNonlin 6.3 (Pharsight, Mountain View, CA, USA) and noncompartmental methods.

Safety assessments
Safety was assessed throughout the study period and for up to 30 (±2) days following the last everolimus dose by reporting the incidence of AEs and serious AEs (SAEs) and their severity and relationship to study drug. Assessments of hematology and blood chemistry and urinalysis were performed at screening, at each of the two baseline visits, and at the end of study treatment. Vital signs, physical condition, ECG, and body weight were regularly assessed. AEs were graded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 4.03.

Statistical analysis
Because the primary statistical analysis did not include hypothesis testing, no power or power-based sample size calculations were performed. The number of subjects enrolled was driven by the targeted precision of 20% of the two-sided 90% confidence interval (CI) for the ratio of geometric means.

The full analysis set population included all randomly assigned participants. The safety population included all participants who received at least one dose of everolimus. The PK analysis set population included all participants who completed at least one treatment period and had evaluable PK data. Evaluable PK data for any treatment period fulfilled the following criteria: no vomiting within 4 hours of dosing, sufficient sample available for analysis, and no use of CYP3A4 inhibitors, inducers, or substrates for 30 days before the first dose until the end of the study.

Data were analyzed for PK and safety using SAS® version 9.1.3 (SAS Institute, Inc., Cary, NC, USA). Descriptive statistics were used to summarize PK parameters, including geometric and arithmetic means, standard deviation (SD), mean and geometric mean coefficient of variation (CV%), median, minimum, and maximum; only median, minimum, and maximum were used for Tmax.

Figure 1 Study design.
Notes: End of pharmacokinetic sampling occurred at days 7 and 21. End of study evaluation was performed 14 (±2) days after everolimus administration in the second time period.
Abbreviation: R, randomization.
Baseline characteristics were comparable between the sequences (Table 1). In the overall population, median age was 47.5 (range, 25 to 65) years, and most participants (73%) were women.

**Pharmacokinetics**

The Cmax of five 1 mg tablets was 48% higher than the Cmax of one 5 mg tablet (geometric mean ratio, 1.48; 90% CI, 1.35–1.62) (Table 2 and Figure 2). Because the 90% CI was not within the boundaries of 0.8–1.25, the everolimus formulations of five 1 mg tablets and one 5 mg tablet did not meet bioequivalence criteria in terms of Cmax. The two formulations had similar AUCinf (geometric mean ratio, 1.08; 90% CI, 1.02–1.16) (Table 2).

Everolimus administered as five 1 mg tablets had a faster absorption rate as demonstrated by a shorter median Tmax than was observed with one 5 mg tablet (0.5 vs 1.0 hour) (Table 3). As assessed by both AUC0–144h and AUCCall, total overall exposure was similar for one 5 mg tablet and five 1 mg tablets (Table 3). No differences in T1/2 and MRT were apparent, suggesting similar distribution and elimination kinetics (Table 3). Mean Vd/F of one 5 mg tablet was higher than that of five 1 mg tablets (Table 3), but the geometric mean ratio and 90% CI suggested no statistically significant difference (ratio, 0.92; 90% CI, 0.85–0.99). Everolimus concentration–time profiles at time points beyond 24 hours postdose were comparable between the two treatment formulations (Figure 3). Similar predose trough concentrations (Cmin) are expected after daily administration of the two formulations.

**Safety**

Overall, 12 (54.5%) participants experienced at least one AE during the study (Table 4). The most frequently reported AEs were headache (27.3%) and nasopharyngitis, nausea, dry skin, and myalgia (9.1% each). Seven (31.8%) participants experienced AEs determined to be related to everolimus, with headache (18.2%) being the most common.

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**Table 1** Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Everolimus</th>
<th>Everolimus</th>
<th>All participants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1×5 mg →</td>
<td>5×1 mg →</td>
<td>N=22</td>
</tr>
<tr>
<td></td>
<td>5×1 mg</td>
<td>5×1 mg</td>
<td></td>
</tr>
<tr>
<td>n=11</td>
<td>n=11</td>
<td>n=11</td>
<td></td>
</tr>
<tr>
<td>Age, years a</td>
<td>50.0 (25–63)</td>
<td>40.0 (27–65)</td>
<td>47.5 (25–65)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>9 (82)</td>
<td>7 (64)</td>
<td>16 (73)</td>
</tr>
<tr>
<td>Men</td>
<td>2 (18)</td>
<td>4 (36)</td>
<td>6 (27)</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>11 (100)</td>
<td>11 (100)</td>
<td>22 (100)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.8 (20.5–28.4)</td>
<td>25.7 (22.3–29.7)</td>
<td>24.5 (20.5–29.7)</td>
</tr>
</tbody>
</table>

*Notes: BMI was calculated using baseline values for weight and height. Values are expressed as median (range). Abbreviation: BMI, body mass index.*

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**Table 2** Relative bioavailability of everolimus one 5 mg tablet versus five 1 mg tablets (PK population, N=22)

<table>
<thead>
<tr>
<th>PK parameter</th>
<th>Everolimus dose</th>
<th>Adjusted geometric mean</th>
<th>Treatment comparison a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Geometric mean ratio</td>
</tr>
<tr>
<td>AUCinf (ng·h/ml)</td>
<td>1×5 mg</td>
<td>243</td>
<td>1.08</td>
</tr>
<tr>
<td></td>
<td>5×1 mg</td>
<td>264</td>
<td></td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>1×5 mg</td>
<td>28.7</td>
<td>1.48</td>
</tr>
<tr>
<td></td>
<td>5×1 mg</td>
<td>42.3</td>
<td></td>
</tr>
</tbody>
</table>

*Note: Comparison of everolimus five 1 mg tablets (test) with one 5 mg tablet (reference). Abbreviations: AUC, area under the concentration–time curve; AUCinf, AUC from time zero to infinity; Cmax, maximum blood concentration; CI, confidence interval; PK, pharmacokinetics.*

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Other AEs suspected to be related to everolimus were nausea, diarrhea, vomiting, oral herpes, myalgia, dizziness, and dry skin. All AEs were grade 1 or 2, and no AE led to study discontinuation. No clinically significant changes in laboratory parameters, vital signs, or ECG intervals were observed during the study.

Discussion
Everolimus efficacy and safety in the oncology setting are well established, and everolimus is currently approved in multiple countries for treatment of patients with various types of cancer. The relationship between dose and the pharmacodynamic response of everolimus in patients with cancer has been established. Following oral administration, everolimus is rapidly absorbed, with a Tmax of 1–2 hours. Steady state is reached by week 2 after daily administration. In addition, Cmin was highly predictive of AUC, with a coefficient of determination of 0.96. A meta-analysis of everolimus in clinical oncology trials demonstrated that a twofold increase in Cmin increased the likelihood of tumor size reduction.

![Figure 2](image_url)  
**Figure 2** Arithmetic mean (SD) blood concentration–time profiles from 0 to 4 hours for everolimus administered as five 1 mg tablets and as one 5 mg tablet (PK population, N=22).

**Abbreviations:** PK, pharmacokinetics; SD, standard deviation.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Summary of secondary PK parameters of everolimus by treatment (PK population, N=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Everolimus treatment</strong></td>
<td><strong>Statistics</strong></td>
</tr>
<tr>
<td>1×5 mg</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td></td>
<td>CV% mean</td>
</tr>
<tr>
<td></td>
<td>Geometric mean</td>
</tr>
<tr>
<td></td>
<td>CV% geometric mean</td>
</tr>
<tr>
<td></td>
<td>Median</td>
</tr>
<tr>
<td></td>
<td>Min, max</td>
</tr>
<tr>
<td>5×1 mg</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td></td>
<td>CV% mean</td>
</tr>
<tr>
<td></td>
<td>Geometric mean</td>
</tr>
<tr>
<td></td>
<td>CV% geometric mean</td>
</tr>
<tr>
<td></td>
<td>Median</td>
</tr>
<tr>
<td></td>
<td>Min, max</td>
</tr>
</tbody>
</table>

**Notes:** For Tmax, only median and range are presented. CV% = SD/mean ×100. CV% geometric mean = sqrt (exp [variance for log-transformed data] – 1) ×100.

**Abbreviations:** AUC0–144h, area under the concentration–time curve from time zero to the 144-hour concentration sampling time; AUCall, area under the concentration–time curve from time zero to time of last observation time point, regardless of whether the last concentration is quantifiable; CL/F, systemic clearance; CV, coefficient of variation (%); Lambda_z, terminal slope of elimination phase; N/A, not applicable; PK, pharmacokinetics; SD, standard deviation; sqrt, square root; T1/2, terminal half-life; Tmax, time taken to reach maximum blood concentration; min, minimum; max, maximum.
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A trend toward reduced risk of progression-free survival events (risk ratio [RR], 0.90; 95% CI, 0.69–1.18) was associated with increased the risk of grade $3$ stomatitis (RR, 1.49; 95% CI, 1.05–2.10) and pulmonary (RR, 1.93; 95% CI, 1.12–3.34) and metabolic (RR, 1.30; 95% CI, 1.02–1.65) events.

Although everolimus is available as a $1$ mg tablet, this formulation has not been evaluated in the oncology setting, and it is not known whether several $1$ mg tablets deliver the same Cmax and bioavailability as a single tablet of a higher dose. Results of this healthy volunteer study, which used a well-established, standard crossover design, suggest that everolimus dosed as five $1$ mg tablets does not have the same bioavailability as one $5$ mg tablet. As assessed by Cmax, the bioequivalence criteria were not met, despite similar AUCinf. The Cmax was 48% higher with five $1$ mg tablets than with one $5$ mg tablet. In addition, Tmax was 0.5 hour with five $1$ mg tablets and 1.01 hours with one $5$ mg tablet. The distribution and elimination kinetics (CL/F, T1/2, and terminal slope of elimination phase parameters) were similar with both everolimus formulations.

The safety profile of everolimus administered as $5$ mg tablets to patients with advanced solid tumors has been well established. Common class effect AEs include mucositis, stomatitis, skin toxicities, pulmonary toxicities, hyperlipidemia, and hyperglycemia. Results of a Phase I dose-escalation study of oral everolimus conducted in patients with advanced solid tumors found dose-limiting toxicities to be stomatitis and fatigue at the $50$ mg once-weekly dosage and hyperglycemia at the $10$ mg daily dosage. Conversely, the safety profile of everolimus administered as several $1$ mg tablets in patients with cancer has not been evaluated. In this single-dose study of healthy volunteers, both formulations were well tolerated, with 12.5% of subjects experiencing at least one AE during the study period, all of which were grade 1 or 2. The most common AE was headache. No serious AEs were reported, no AE led to treatment discontinuation, and no new safety concerns were identified.

Table 4 Number of subjects who experienced adverse events, regardless of study drug relationship (safety population, N=22)

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>All subjects (N=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>12 (54.5)</td>
</tr>
<tr>
<td>Headache</td>
<td>6 (27.3)</td>
</tr>
<tr>
<td>Dry skin</td>
<td>2 (9.1)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>2 (9.1)</td>
</tr>
<tr>
<td>Nasopharyngitis</td>
<td>2 (9.1)</td>
</tr>
<tr>
<td>Nausea</td>
<td>2 (9.1)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>Erythema</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>Oral herpes</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>Phlebitis</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>Rhinitis</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>Vaginal hemorrhage</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1 (4.5)</td>
</tr>
</tbody>
</table>

Note: All adverse events were grade 1 or 2.
In conclusion, everolimus formulations of one 5 mg tablet and five 1 mg tablets did not meet bioequivalence criteria in terms of Cmax despite similar AUCinf in this Phase I study of healthy volunteers. In addition to the consideration that the safety profile of several 1 mg tablets in patients with advanced solid tumors is unknown, this finding suggests that these formulations are not interchangeable.

Acknowledgments
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Disclosure
Drs Thudium, Eddy, and Cheung are employees of Novartis Pharmaceuticals Corporation. Drs Gallo, Bouillaud, and Sachs are employees of Novartis Pharma AG.

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